

AD-A033 403

ARMED FORCES RADIOBIOLOGY RESEARCH INST BETHESDA MD
ETHANOL-INDUCED DEPLETION OF CEREBELLAR GUANOSINE 3',5'-CYCLIC --ETC(U)
JUN 76 J D REDOS, G N CATRAVAS, W A HUNT
AFRRI-SR76-24

F/G 6/1

UNCLASSIFIED

NL

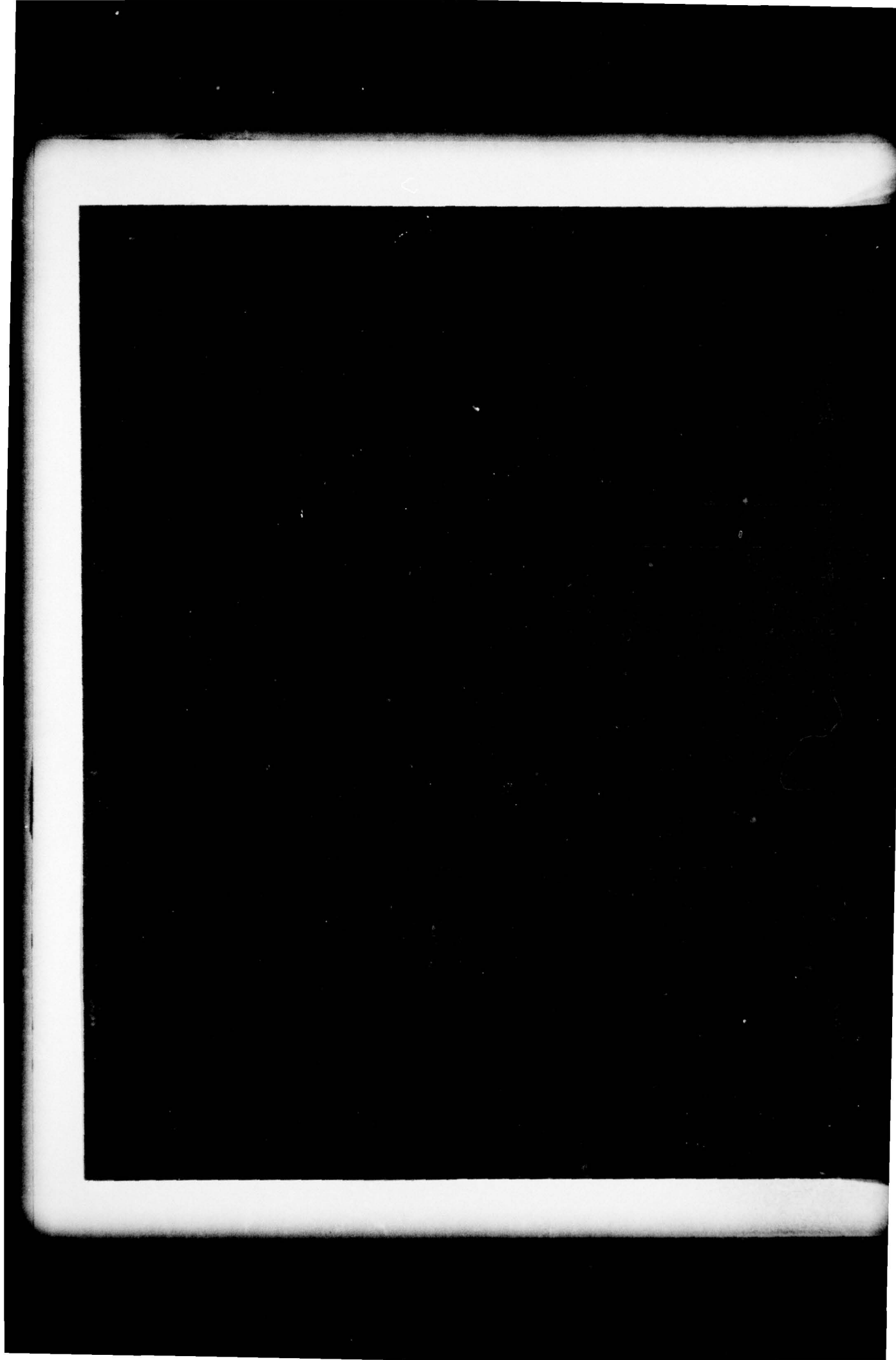
| OF |
ADA033403



END

DATE
FILMED
2 - 77

ADA033403



UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AFRRI-SR76-24	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) ETHANOL-INDUCED DEPLETION OF CEREBELLAR GUANOSINE 3',5'-CYCLIC MONOPHOSPHATE LEVELS.		5. TYPE OF REPORT & PERIOD COVERED
7. AUTHOR(s) J. D. Redos,* G. N. Catravas and W. A. Hunt (*American University)		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS Armed Forces Radiobiology Research Institute Defense Nuclear Agency Bethesda, Maryland 20014		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS Director Defense Nuclear Agency (DNA) Washington, D. C. 20305		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NWED QAXM C 912 18
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) Scientific rept,		12. REPORT DATE June 1976
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		13. NUMBER OF PAGES 13
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		15. SECURITY CLASS. (of this report) UNCLASSIFIED
18. SUPPLEMENTARY NOTES		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Single doses of ethanol induce a severe depletion (95 percent) of cerebellar guanosine 3',5'-cyclic monophosphate (c-GMP) levels within 1 hour after adminis- tration. The degree of this depletion is a function of the amount of ethanol in the blood. Interactions between ethanol and c-GMP may account for some of the intox- icating properties of ethanol. The results of this study provide further information		

DD FORM 1 JAN 73 1473

EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-014-6601

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

034 700

mt

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

20. ABSTRACT (continued)

→ on the development and exploration of models for chronic insults to the brain, such as long-term exposure to toxic chemicals and ionizing and nonionizing radiation.

A

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

TABLE OF CONTENTS

	Page
Introduction	3
Methods	3
Results and Discussion	4
References	7

FIGURE

Figure 1. Cerebellar c-GMP levels after a single dose of ethanol as a function of time after treatment and the blood ethanol concentration	5
--	---

TABLE

Table 1. Cerebellar c-GMP and c-AMP Levels 2 Hours after a Single Dose of Ethanol	4
---	---

ADDRESSING UNIT	
RTIS	<input checked="" type="checkbox"/>
BOD	<input type="checkbox"/>
UNANNOUNCED	<input type="checkbox"/>
JUSTIFICATION	
BY	
DISTRIBUTION/AVAILABILITY CODE	
DISC.	STATE, NITR. CODE
A	

INTRODUCTION

The role of guanosine 3',5'-cyclic monophosphate (c-GMP) in neuronal function is just beginning to be understood. c-GMP may be a mediator of the actions of acetylcholine in the superior cervical ganglion,⁹ cerebral cortex^{14,20} and cerebellum¹² and may play an additional role as an antagonist of the actions of adenosine 3',5'-cyclic monophosphate (c-AMP).¹¹ The relationship of cyclic nucleotides to neuroexcitability has prompted several investigations of the effects of ethanol on the c-AMP system.^{7,10,13,22} To date there have been no reports of the effect of ethanol on c-GMP levels. Our initial studies reported here indicate that cerebellar c-GMP levels are severely depleted after a single dose of ethanol.

METHODS

Male Sprague-Dawley rats (200-300 g) received 6 g/kg of ethanol as a 20 percent (w/v) aqueous solution of absolute ethanol by means of intragastric intubation. At various times after treatment the animals were euthanatized by focused microwave irradiation (Litton Menu-master 70/50, modified by Medical Engineering Consultants, Lexington, Massachusetts; 1.3 kW; 3.5-sec exposure time), the brains excised and the cerebella removed for analysis. The tissue was purified as outlined by Mao and Guidotti.¹⁵ Brain samples were extracted with 1 ml of 0.4 N perchloric acid, neutralized with 0.15 ml of 3 M Tris and centrifuged at 30,000 x g for 20 minutes. The supernatant solutions were purified by first passing them through an alumina column (0.4 x 4.3 cm) equilibrated with 0.06 M Tris-HCl buffer (pH 7.5). The cyclic nucleotides were eluted with 0.6 M Tris-HCl (pH 7.5) onto a Dowex 1 x 2 column (0.4 x 8.0 cm, 200-400 mesh) equilibrated with water. After washing the resin with 4 ml of water c-AMP was eluted with 3 ml of 0.05 N HCl and c-GMP with 3 ml of 0.5 N HCl. The c-AMP fraction was neutralized with 0.2 ml of 0.6 M Tris base and passed through a Dowex 50W-X8 column (0.4 x 3.0 cm, 200-400 mesh H⁺ form) equilibrated with water. After washing the resin with 2 ml of water, the c-AMP was eluted with another 2.5 ml of water. The c-GMP fraction was lyophilized, rehydrated in 1 ml of water and passed through a Sephadex G-10 column (0.4 x 7.5 cm) equilibrated with 0.1 mM NH₄OH, then

water. The c-GMP was collected in 2 ml of water and lyophilized. c-AMP was quantitated using the competitive protein binding assay of Gilman,⁸ while c-GMP was measured by the binding assay of Murad et al.¹⁸ as modified by Dinnendahl.⁴ Blood ethanol concentrations were determined enzymatically using the Calbiochem Ethanol Stat-Pack. Statistical comparisons were made using Student's "t" test.

RESULTS AND DISCUSSION

A single dose of ethanol resulted in a rapid depletion of cerebellar c-GMP. c-GMP levels were reduced 80 percent 2 hours after treatment (Table 1) with no alteration in c-AMP levels. On closer examination c-GMP was observed to be depleted

Table 1. Cerebellar c-GMP and c-AMP Levels 2 Hours after a Single Dose of Ethanol (6 g/kg, p.o.). Each value represents the mean \pm standard error and was obtained from five to ten animals. *Denotes statistical significance ($P < 0.05$).

	c-GMP (pmoles/mg of protein)	c-AMP (pmoles/mg of protein)
Controls	4.0 \pm 0.14	3.4 \pm 0.16
Ethanol-treated	0.5 \pm 0.04 *	3.2 \pm 0.08

maximally by 95 percent 1 hour after ethanol administration (Figure 1). Over a 30-hour period c-GMP levels slowly returned to control levels in parallel with blood ethanol elimination.

To our knowledge, ethanol is one of the most effective compounds capable of depleting c-GMP in the cerebellum. The potential significance of this finding depends on the role of c-GMP in the function of the cerebellum. Little information is known about the location of c-GMP, but experiments with mutant mice deficient in different cell types suggest that c-GMP is localized in Purkinje cells.¹⁷ Purkinje cells act in the proper control of body musculature by providing inhibitory input to other areas of the brain and derive their excitatory input from climbing and mossy fibers projecting

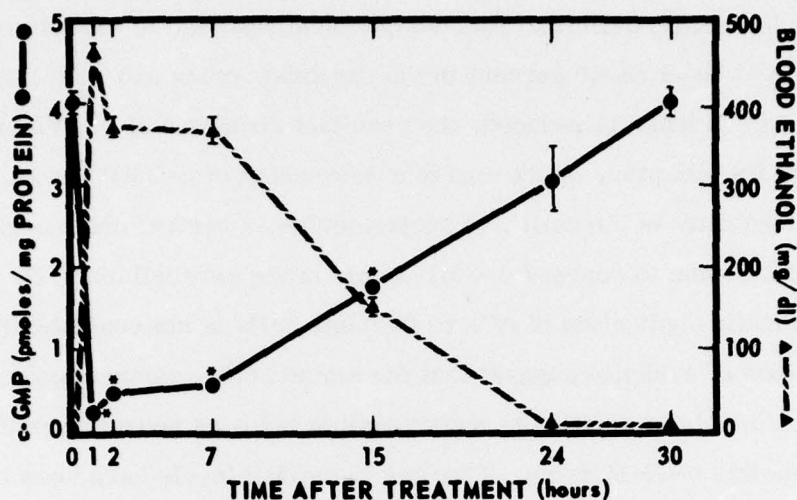


Figure 1. Cerebellar c-GMP levels after a single dose of ethanol (6 g/kg) as a function of time after treatment and the blood ethanol concentration. Each value represents the mean \pm standard error and was obtained from five to ten animals. *Denotes statistical significance ($P < 0.05$).

from the brain stem.¹ Ionophoretic application of c-GMP to Purkinje cells suggests that it mediates excitatory influences.¹⁹

The mechanism by which ethanol depletes cerebellar c-GMP is unknown. However, several possibilities exist. Ethanol could directly either inhibit guanylate cyclase, the enzyme that converts guanosine triphosphate to c-GMP, or stimulate cyclic nucleotide phosphodiesterase, the enzyme that metabolizes c-GMP to guanosine monophosphate. In studies of the effects of ethanol on phosphodiesterase neither a single dose of ethanol (4 g/kg, i.p.) nor chronic ingestion for up to 3 weeks had any effect on phosphodiesterase activity.^{7,10,13,22} The effects of ethanol on guanylate cyclase have not been reported.

An alternative explanation of these results might involve an effect of ethanol on putative neurotransmitters thought to be interacting with the c-GMP system. Acetylcholine has been shown to stimulate the formation of c-GMP in the superior cervical ganglion,⁹ cerebral cortex^{14,20} and cerebellum,¹² an effect which can be blocked by

atropine, a cholinergic antagonist. This suggests that c-GMP levels may in part be regulated by cholinergic activity. Ethanol has been reported to inhibit acetylcholine release in vivo as much as 60 percent in the cerebral cortex and reticular formation.⁵ If cerebellar ACh release is reduced, the resultant reduction of the interaction of acetylcholine with its receptor, might lead to a depression of c-GMP levels. Further support of this possibility is the ability of oxotremorine, a central cholinergic agonist, to elevate and of atropine to depress c-GMP levels in the cerebellum.⁶ On the other hand, iontophoretic application of ACh to Purkinje cells is not consistently excitatory.¹⁹

Other lines of evidence suggest that the amino acids, gamma-aminobutyric acid (GABA) and glutamate in addition to their possible roles as neurotransmitters, may regulate cerebellar c-GMP levels. Changes in c-GMP levels have been reported to be positively correlated to glutamate levels, while negatively correlated to GABA levels.¹⁶ Glutamate is excitatory and GABA inhibitory to Purkinje cells.^{3,19} Single doses of ethanol have no effect on cerebellar GABA, but reduce glutamate levels 15-20 percent.²¹ The degree to which glutamate release may be inhibited cannot be deduced from these data. In any event, alterations in the actions of glutamate might explain in part the ethanol-induced c-GMP depletion. Whether disturbances in the interaction of c-GMP and neurotransmitters are in any way responsible for some of the biochemical effects of ethanol will depend on further research into the nature of these interactions.

It appears from Figure 1 that at blood ethanol concentrations encountered with moderate drinking (50-150 mg/dl) a significant reduction of cerebellar c-GMP occurs. How c-GMP is related to the intoxicating properties of ethanol is yet to be determined. A major neurological decrement of ethanol intake is lack of muscular coordination. Since insufficient excitatory input to Purkinje cells can lead to ataxia,² disruption of the actions of c-GMP in its role as a possible mediator of excitatory influences might explain in part the ataxia observed after drinking alcoholic beverages.

REFERENCES

1. Bloedel, J. R. Cerebellar afferent systems: a review. *Prog. Neurobiol.* 2: 3-68, 1973.
2. Brookhart, J. M. The cerebellum. In: *Handbook of Physiology, Section 1: Neurophysiology*, Field, J., editor, Vol. 2, pp. 1245-1280. Baltimore, Maryland, Waverly Press, 1960.
3. Chujo, T., Yamada, Y. and Yamamoto, C. Sensitivity of Purkinje cell dendrites to glutamic acid. *Exp. Brain Res.* 23:293-300, 1975.
4. Dinnendahl, V. A rapid and simple procedure for the determination of guanosine 3',5'-monophosphate by use of the protein-binding method. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 284:55-61, 1974.
5. Erickson, C. K. and Graham, D. T. Alteration of cortical and reticular acetylcholine release by ethanol in vivo. *J. Pharmacol. Exp. Ther.* 185:583-593, 1973.
6. Ferrendelli, J. A., Steiner, A. L., McDougal, D. B., Jr. and Kipnis, D. M. The effect of oxotremorine and atropine on cGMP and cAMP levels in mouse cerebral cortex and cerebellum. *Biochem. Biophys. Res. Commun.* 41:1061-1071, 1970.
7. French, S. W., Palmer, D. S., Narod, M. E., Reid, P. E. and Ramey, C. W. Noradrenergic sensitivity of the cerebral cortex after chronic ethanol ingestion and withdrawal. *J. Pharmacol. Exp. Ther.* 194:319-326, 1975.
8. Gilman, A. G. A protein binding assay for adenosine 3':5'-cyclic monophosphate. *Proc. Natl. Acad. Sci. USA* 67:305-312, 1970.
9. Greengard, P. and Kebabian, J. W. Role of cyclic AMP in synaptic transmission in the mammalian peripheral nervous system. *Fed. Proc.* 33:1059-1067, 1974.
10. Israel, M. A., Kimura, H. and Kuriyama, K. Changes in activity and hormonal sensitivity of brain adenyl cyclase following chronic ethanol administration. *Experientia* 28:1322-1323, 1972.
11. Kolata, G. B. Cyclic GMP: cellular regulatory agent? *Science* 182:149-151, 1973.

12. Kuo, J.-F., Lee, T.-P., Reyes, P. L., Walton, K. G., Donnelly, T. E., Jr., and Greengard, P. Cyclic nucleotide-dependent protein kinases. X. An assay method for the measurement of guanosine 3',5'-monophosphate in various biological materials and a study of agents regulating its levels in heart and brain. *J. Biol. Chem.* 247:16-22, 1972.
13. Kuriyama, K. and Israel, M. A. Effect of ethanol administration on cyclic 3',5'-adenosine monophosphate metabolism in brain. *Biochem. Pharmacol.* 22:2919-2922, 1973.
14. Lee, T.-P., Kuo, J. F. and Greengard, P. Role of muscarinic cholinergic receptors in regulation of guanosine 3':5'-cyclic monophosphate content in mammalian brain, heart muscle, and intestinal smooth muscle. *Proc. Natl. Acad. Sci. USA* 69:3287-3291, 1972.
15. Mao, C. C. and Guidotti, A. Simultaneous isolation of adenosine 3',5'-cyclic monophosphate (cAMP) and guanosine 3',5'-cyclic monophosphate (cGMP) in small tissue samples. *Anal. Biochem.* 59:63-68, 1974.
16. Mao, C. C., Guidotti, A. and Costa, E. The regulation of cyclic guanosine monophosphate in rat cerebellum: possible involvement of putative amino acid neurotransmitters. *Brain Res.* 79:510-514, 1974.
17. Mao, C. C., Guidotti, A. and Landis, S. Cyclic GMP: reduction of cerebellar concentrations in "nervous" mutant mice. *Brain Res.* 90:335-339, 1975.
18. Murad, F., Manganillo, V. and Vaughan, M. A simple, sensitive protein-binding assay for guanosine 3':5'-monophosphate. *Proc. Natl. Acad. Sci. USA* 68:736-739, 1971.
19. Siggins, G. R., Henriksen, S. J. and Landis, S. C. Effects of iontophoresis of neurotransmitters and cyclic nucleotides and of stimulation of the locus coeruleus (LC) on Purkinje neurons (PNs) of the weaver mutant mouse. In: *Neuroscience Abstracts, 5th Annual Meeting of the Society for Neuroscience, New York City, November 2-6, 1975, Vol. 1, p. 378.* Bethesda, Maryland, Society for Neuroscience, 1975.
20. Stone, T. W., Taylor, D. A. and Bloom, F. E. Cyclic AMP and cyclic GMP may mediate opposite neuronal responses in the rat cerebral cortex. *Science* 187:845-847, 1975.
21. Sytinsky, I. A., Guzikov, B. M., Gomanko, M. V., Eremin, V. P. and Konovalova, N. N. The gamma-aminobutyric acid (GABA) system in brain during acute and chronic ethanol intoxication. *J. Neurochem.* 25:43-48, 1975.

22. Volicer, L. and Gold, B. I. Effect of ethanol on cyclic AMP levels in the rat brain. *Life Sci.* 13:269-280, 1973.